

We claim:

1. A composition comprising a placental stem cell isolated from the amnion wherein the composition is enriched for cells that express at least one marker selected from the group consisting of c-kit, Thy-1, Oct-4, Nanog, SOX2, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Lefty-A, FGF-4, Rex-1 and TDGF-1.
2. A composition comprising a placental stem cell which is isolated from the amniotic epithelium and enriched for cells that express at least one marker selected from the group consisting of c-kit, Thy-1, Oct-4, Nanog, SOX2, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Lefty-A, FGF-4, Rex-1 and TDGF-1.
3. A composition comprising a placental stem cell which is isolated from amniotic mesenchyme and enriched for cells that express Oct-4 or SOX2.
4. The composition of any one of Claims 1 to 3, which is enriched for cells that express Nanog and Oct-4.
5. The composition of any one of Claims 1 to 4, which is enriched for cells that express one or more cytokeratins.
6. The composition of Claim 5, wherein the cells express one or more cytokeratins selected from the group consisting of AE1, AE3, cytokeratin 8, cytokeratin 18, and cytokeratin 19.
7. The composition of any one of Claims 1 to 6, wherein the cells are negative for CD34 expression.
8. The composition of any one of Claims 1 to 7, wherein the stem cell is recombinantly engineered to express a heterologous protein.
9. A pharmaceutical composition comprising the composition of any one of Claims 1 to 9 and a physiologically acceptable carrier.
10. A composition comprising a cultured proliferating placental stem cell isolated from the amnion which expresses at least one marker selected from the group consisting of c-kit, Thy-1, Oct-4, Nanog, SOX-2, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Lefty A, FGF-4, Rex-1 and TDGF-1.
11. The composition of Claim 10, which is effective to support proliferation of the placental stem cell for greater than 11 days.
12. The composition of Claim 10, wherein the placental stem cell is isolated from amniotic epithelium.

13. The composition of Claim 12, wherein the composition is enriched for cells expressing at least one marker selected from the group consisting of c-kit, Thy-1, Oct-4, Nanog, SOX-2, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Lefty A, FGF-4, Rex-1 and TDGF-1.

14. The composition of Claim 10, wherein the placental stem cell is isolated from amniotic mesenchyme and expresses at least one marker selected from the group consisting of Oct-4, Nanog, and SOX2.

15. The composition of Claim 14, wherein the composition is enriched for cells expressing at least one marker selected from the group consisting of Oct-4, Nanog, and SOX2.

16. The composition of Claim 10, which is enriched for cells that express one or more cytokeratins.

17. The composition of Claim 16, wherein the cells express one or more cytokeratins selected from the group consisting of AE1, AE3, cytokeratin 8, cytokeratin 18, and cytokeratin 19.

18. The composition of Claim 10, wherein the placental stem cell is negative for CD34 expression.

19. The composition of Claim 10, wherein the placental stem cell is engineered to express a therapeutic protein.

20. The composition of Claim 10, wherein the placental stem cell is cultured with a growth factor, a hormone, or a cytokine.

21. The composition of Claim 10 wherein the placental stem cell is cultured with EGF.

22. The composition of Claim 10 wherein the placental stem cell is cultured with TGF- α .

23. A method of making the composition of Claim 1, which comprises the step of selecting a placental stem cell isolated from the amnion which expresses at least one marker selected from the group consisting of c-kit, Thy-1, Oct-4, Nanog, SOX2, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Lefty A, FGF-4, Rex-1 and TDGF-1.

24. The method of Claim 23, wherein the placental cell is isolated from the amniotic epithelium.

25. The method of Claim 23, wherein the cell isolated from the amnion is isolated from the amniotic mesenchyme and is selected for expression of at least one marker selected from the group consisting of Oct-4, Nanog, and SOX2.

26. The method of Claim 23, wherein the selecting uses antibodies to at least one marker selected from the group consisting of c-kit, Thy-1, Oct-4, Nanog, SOX2, SSEA-3, SSEA-4, TRA1-60, TRA1-81, Lefty A, FGF-4, Rex-1 and TDGF-1.

27. The method of Claim 23, wherein the placental stem cells are cultured and proliferated with a cytokine effective to support proliferation of the placental stem cells for greater than 11 days.

28. The method of Claim 23, wherein the placental stem cells are cultured and proliferated with a growth factor, a hormone, or a cytokine.

29. The method of Claim 23, wherein the placental stem cells are cultured and proliferated with EGF.

30. The method of Claim 23, wherein the placental stem cell is cultured with TGF- α .

31. The method of Claim 23, wherein the selecting includes density separation of cells from the amnion that express stem cell markers.

32. The method of Claim 31, wherein the separation is on Percoll.

33. The method of Claim 23, wherein the selecting includes collection of non-adherent amniotic epithelial cells.

34. The method of Claim 33, wherein the non-adherent amniotic epithelial cells are cultured on a monolayer of adherent amniotic epithelial cells.

35. The method of Claim 23, wherein the placental stem cells are cultured in an anchorage-independent manner.

36. The method of Claim 23, wherein the placental stem cells are cultured under subatmospheric ambient oxygen conditions.

37. The method of Claim 36, wherein the subatmospheric ambient oxygen conditions comprise an oxygen concentration of between about 0.25% and 15%.

38. The method of Claim 36, wherein the subatmospheric ambient oxygen conditions comprise an oxygen concentration of between about 2% and 10%.

39. The method of Claim 36, wherein the subatmospheric ambient oxygen conditions comprise an oxygen concentration of about 5%.

40. A method of making a cardiomyocyte comprising culturing a stem cell of any one of Claims 1-21 in a media that contains an appropriate amount of ascorbic acid 2-phosphate for a sufficient period of time to allow the stem cell to differentiate into a cardiomyocyte.

41. A cardiomyocyte obtained from the method of Claim 40, which expresses at least one marker selected from the group consisting of: MLC-2A, MLC-2V, hANP, cTnT, alpha-actinin, GATA-4 and Nkx 2.5.

42. A pharmaceutical composition comprising an effective amount of a cardiomyocyte of Claim 41 and a pharmaceutically acceptable carrier.

43. A method of determining whether a test agent is toxic to a cardiomyocyte, comprising contacting the cardiomyocyte of Claim 41 with an appropriate amount of the test agent for a time sufficient for a toxic effect on the cardiomyocyte to be detected, and determining whether the test agent has a toxic effect on the cardiomyocyte.

44. A method of determining a metabolic product of a test agent comprising contacting the cardiomyocyte of Claim 41 with an appropriate amount of the test agent for a time sufficient for the test agent to be metabolized, and detecting the presence of the metabolized product.

45. A method of making a hepatocyte comprising culturing a stem cell of any one of Claims 1 to 21 in a media that comprises an appropriate amount of dexamethasone, ITS, EGF, FGF-2, FGF-4, FGF-7, HGF, phenobarbital, Type-I collagen or a combination thereof for a sufficient period of time to allow the stem cell to differentiate into a hepatocyte.

46. A method of making a hepatocyte comprising:

- a) culturing a placental stem cell of any one of Claims 1 to 21 to induce differentiation to a mesendodermal lineage cell;
- b) culturing the mesendodermal lineage cell of step (a) to induce hepatic differentiation.

47. The method of Claim 46, wherein step (a) comprises culturing the placental stem cell in a media that comprises an appropriate amount of activin A or BMP-4 effective to induce differentiation to a mesendodermal cell.

48. The method of Claim 46, wherein step (b) comprises:

- i) culture of the mesendodermal cell of step (a) in a media that comprises an appropriate amount of FGF-8, TGF-beta, FGF-19, HGF, EGF, dexamethasone, or a combination thereof, followed by:
- ii) culture in a media that comprises an appropriate amount of oncostatin M, beta-naphtholflavone, vitamin K₂, or a ligand for peroxisome proliferator activated receptor (PPAR), pregnane X receptor (PXR) or constitutive androstane receptor (CAR).

49. The method of Claim 45, wherein the media comprises an appropriate amount of EGF and dexamethasone.

50. The method of Claim 45, wherein the media comprises an appropriate amount of EGF, dexamethasone, and insulin and the stem cell is cultured on a collagen containing substrate.

51. The method of Claim 50, wherein the media further contains phenobarbital.

52. A hepatocyte obtained from the method of any one of Claims 45 to 51, which expresses at least one marker selected from the group consisting of: albumin, CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP3A4, AFP, A1AT, HNF1, HNF4 and C/EBP alpha.

53. A pharmaceutical composition comprising an effective amount of a hepatocyte of Claim 52 and a pharmaceutically acceptable carrier.

54. A method of determining whether a test agent is toxic to a hepatocyte comprising contacting the hepatocyte of Claim 52 with an appropriate amount of the test agent for a time sufficient for a toxic effect on the hepatocyte to be detected, and determining whether the test agent has a toxic effect on the hepatocyte.

55. A method of determining a metabolic product of a test agent comprising contacting the hepatocyte of Claim 52 with an appropriate amount of the test agent for a time sufficient for the test agent to be metabolized, and detecting the presence of the metabolized product.

56. A method of making a pancreatic cell comprising culturing a stem cell of any one of Claims 1 to 21 in a media that comprises an appropriate amount of nicotinamide, dexamethasone, ITS, or a combination thereof for a sufficient period of time to allow the stem cell to differentiate into a pancreatic cell.

57. The method of Claim 56, wherein the stem cell is cultured on a Matrigel coated substrate.

58. A pancreatic cell obtained from the method of any one of Claims 56 to 57, which expresses at least one marker selected from the group consisting of Pax6, Pdx1, insulin, glucagon, and Nkx2.2.

59. A pharmaceutical composition comprising an effective amount of a pancreatic cell of Claim 58 and a pharmaceutically acceptable carrier.

60. A method of determining whether a test agent is toxic to a pancreatic cell comprising contacting the pancreatic cell of Claim 58 with an appropriate amount of the test agent for a time sufficient for a toxic effect on the pancreatic cell to be detected, and determining whether the test agent has a toxic effect on the pancreatic cell.

61. A method of determining a metabolic product of a test agent comprising contacting the pancreatic cell of Claim 58 with an appropriate amount of the test agent for a

time sufficient for the test agent to be metabolized, and detecting the presence of the metabolized product.

62. A method of making a neural cell comprising culturing a stem cell of any one of Claims 1 to 21 in a media that contains an appropriate amount of trans-retinoic acid or FGF-4 for a sufficient period of time to allow the stem cell to differentiate into a neural cell.

63. A neural cell obtained from the method of Claim 62, which expresses at least one marker selected from the group consisting of GFAP, CNP, beta-tubulin III, Nestin, GAD, NSE, NF-M and MBP.

64. A pharmaceutical composition comprising an effective amount of a neural cell of Claim 63 and a pharmaceutically acceptable carrier.

65. A method of determining whether a test agent is toxic to a neural cell comprising contacting the neural cell of Claim 63 with an appropriate amount of the test agent for a time sufficient for a toxic effect on the neural cell to be detected, and determining whether the test agent has a toxic effect on the neural cell.

66. A method of determining a metabolic product of a test agent comprising contacting the neural cell of Claim 63 with an appropriate amount of the test agent for a time sufficient for the test agent to be metabolized, and detecting the presence of the metabolized product.

67. A method of making a vascular endothelial cell comprising culturing a stem cell of any one of Claims 1 to 21 on a Matrigel coated substrate for a sufficient period of time to allow the stem cell to differentiate into a vascular endothelial cell.

68. The method of Claim 67, wherein the stem cell is cultured in a media that contains an effective amount of FGF-4 or trans-retinoic acid.

69. A vascular endothelial cell obtained from the method of any one of Claims 67 to 68, which expresses the FLT-1 marker.

70. A pharmaceutical composition comprising an effective amount of a vascular endothelial cell of Claim 69 and a pharmaceutically acceptable carrier.

71. A method of determining whether a test agent is toxic to a vascular endothelial cell comprising contacting the vascular endothelial cell of Claim 69 with an appropriate amount of the test agent for a time sufficient for a toxic effect on the vascular endothelial cell to be detected, and determining whether the test agent has a toxic effect on the vascular endothelial cell.

72. A method of determining a metabolic product of a test agent comprising contacting the vascular endothelial cell of Claim 69 with an appropriate amount of the test

agent for a time sufficient for the test agent to be metabolized, and detecting the presence of the metabolized product.

73. A method of regenerating or restoring a metabolic function to a patient in need thereof comprising the step of administering an effective amount of the composition of any one of Claims 1 to 21.

74. The method of Claim 73, wherein the composition comprising placental stem cells is differentiated into cardiomyocytes prior to administration.

75. The method of Claim 73, wherein the composition comprising placental stem cells is differentiated into hepatocytes prior to administration.

76. The method of Claim 73, wherein the composition comprising placental stem cells is differentiated into pancreatic cells prior to administration.

77. The method of Claim 73, wherein the composition comprising placental stem cells is differentiated into neural cells prior to administration.

78. The method of Claim 73, wherein the composition comprising placental stem cells is differentiated into vascular endothelial cells prior to administration.

79. The method of any one of Claim 73 to 78, wherein the composition comprising placental stem cells is administered by injection.

80. The method of any one of Claim 73 to 78, wherein the composition comprising placental stem cells is administered systemically.

81. The method of any one of Claim 73 to 78, wherein the composition comprising placental stem cells is administered into an organ.

82. The method of any one of Claim 73 to 78, wherein the composition comprising placental stem cells is incorporated into a support matrix.

83. A method for humanizing an animal organ comprising transplanting a placental stem cell composition of any one of Claims 1 to 21 into an animal organ, wherein the placental stem cell composition is a human placental stem cell composition, and the stem cells regenerate and repopulate the animal organ.

84. The method of Claim 83, wherein the animal organ is depleted of native cells prior to transplant.

85. The method of Claim 83, wherein the organ is selected from the group consisting of liver, pancreas, brain and heart.

86. The method of Claim 83, wherein the organ is liver and the placental stem cells are transplanted by administration into the spleen.